IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Masataka KUWANA, et al.		
		Group Art Unit:	1649
Serial No.:	10/549,707		
		Examiner:	Dutt, Aditi
Filed:	October 27, 2005		
		Confirmation:	2198
For:	MONOCYTE-ORIGIN MULTIPOTENT CELL MOMC		

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is a Declaration under 37 C.F.R. §1.132 by Masataka Kuwana, MD, Ph.D. in the above-identified application.

I, the undersigned, Masataka Kuwana, declare and state that:

1. I am a co-inventor of the subject patent application having serial no. 10/549,707.

2. My education and professional experience as an expert in the area of tissue engineering are set forth on the attached copy of my Curriculum Vitae.

3. I have read and understand U.S. Patent Application Serial No. 10/549,707, entitled "MONOCYTE-ORIGIN MULTIPOTENT CELL MOMC," and I submit this Declaration in its support.

4. I have read and understand the August 10, 2007 Final Official Action issued in the above-identified case.

5. I have read and understand the publication of Zhao, et al. (*PNAS*, 100: 2426-2431, 2003) cited by the Examiner.

6. In particular, I understand that in the August 10, 2007 Final Official Action, the Examiner has rejected claims 2-8 because they are anticipated by Zhao, et al. Specifically, the Examiner states that the Zhao, et al. reference teaches the isolation of pluripotent stem cells (PSC) from human peripheral blood monocytes that resemble fibroblasts and express the monocytic and hematopoietic cellular differentiation stem cell markers, such as CD14, CD34 and CD45. The Zhao, et al. reference allegedly further discloses that human peripheral blood cells containing monocytes, when cultured under specific conditions, differentiate into macrophages, lymphocytes, epithelial, neuronal, endothelial and hepatocytes (Final Office Action- pages 3-6). As a person skilled in the art, I respectfully disagree with the Examiner's rejection.

7. The inventors of the instant application attempted the differentiation induction of MOMC into T-cells using IL-2, as described in Zhao, et al. The expression of CD3 was analyzed with a flow cytometry technique. The results, as shown in Figure 1 below, demonstrate that CD3 was not expressed. Thus, MOMC does not differentiate into T-cell using the method described in Zhao, et al.. Furthermore, the inventors attempted the differentiation induction of MOMC into neuronal cells, epithelial cells and hepatocytes using NGF, EGF and HGF, respectively. When MOMC was immunostained with an immunoenzymatic method, no brown coloration of MOMC was observed. As shown in Figure 1 below, MAP2 (a marker of neuronal cells), keratin (a marker of epithelial cells), and albumin (a marker of hepatocytes), were not

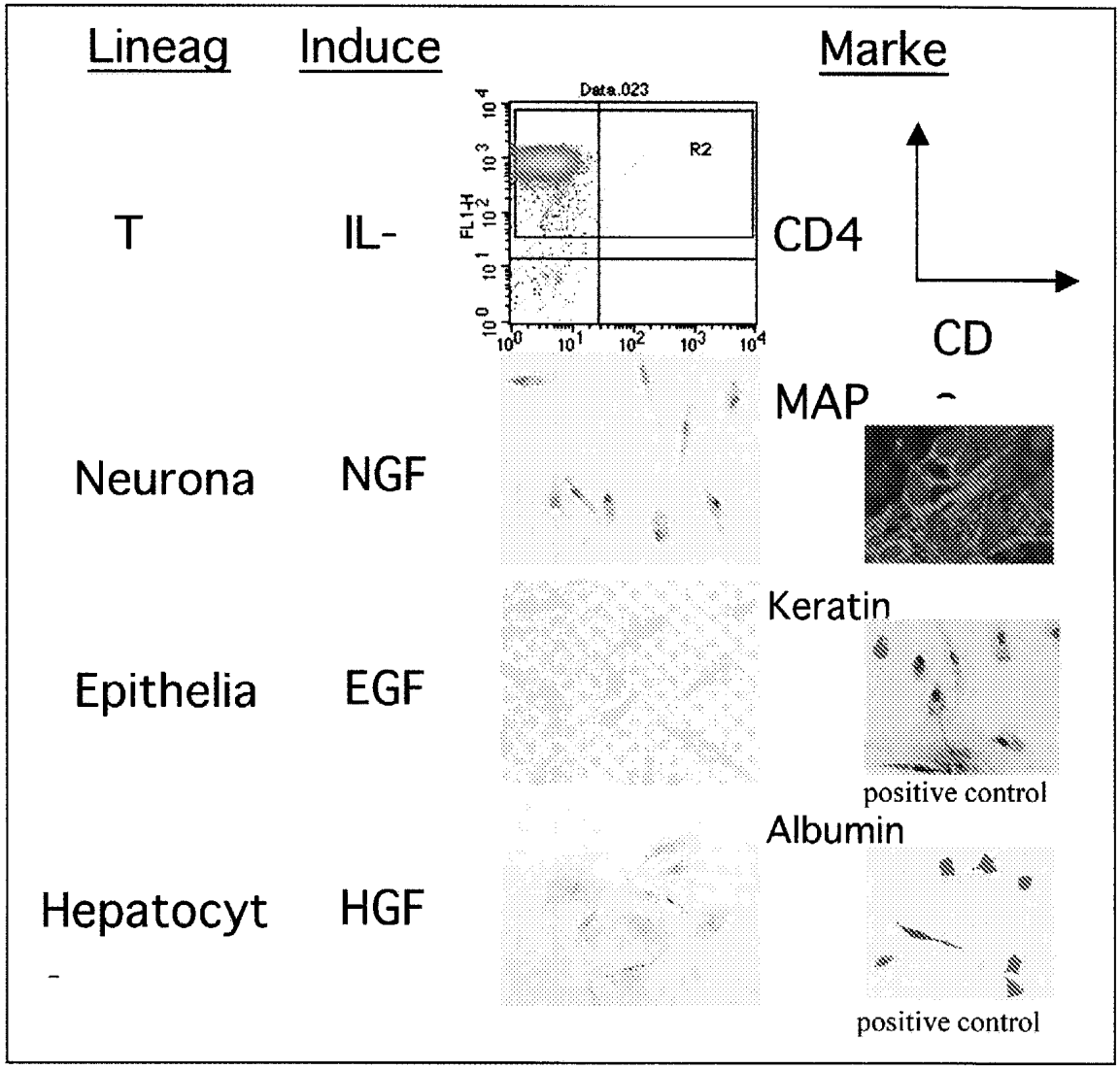

Figure 1: Results of experiments concerning differentiation abilities of human MOMC cultured under the differentiation conditions of PSC into T-cells, neuronal cells, epithelial cells and hepatocytes.

expressed. Hence, it was shown that MOMC does not differentiate into neuronal cells, epithelial cells or hepatocytes. These results show that MOMC are clearly distinct from PSC.

8. Finally, the inventors have also carried out a check experiment, and the results of Zhao, et al. were not reproducible[1]. The steps of the check experiments carried out by the

[1] Reproducibility is solely based on the disclosure of Zhao, et al. and does not mean that there is no reproducibility in Zhao, et al. when special techniques or materials are used but not disclosed in their article.

inventors are set forth here. Monocytes were cultured according to the method of Zhao, et al.(with medium containing M-CSF and LIF), and cells morphologically resembling fibroblasts were observed. However, the frequency of the "cells that morphologically resembled fibroblasts" was much lower than that described in Zhao, et al., and though their cloning was attempted through the method described in Zhao, et al, the cells did not proliferate and clone. The data, therefore, which should be obtained from their separation, purification and analysis were not available. Moreover, their flow cytometry analysis showed a slight expression of CD34 in the cells, which is within the margin of error of flow cytometry analysis. The expression of CD34 was not detected with either immunostaining or the RT-PCR method as shown in Zhao, et al. Furthermore, the inventors confirmed that the cells cultured according to the method of Zhao, et al., which include the "cells that morphologically resembled fibroblasts," did not express CD3 in the presence of IL-2, vWF in the presence of EGF, or AFP in the presence of HGF. These cells also did not differentiate into osteoblasts, skeletal myoblasts or chondrocytes under the differentiation induction conditions of MOMC set forth in the instant application.

9. Zhao et al. disclose pluripotent stem cells (PSC) which express CD14, CD34 and CD45. Zhao et al. also describe that PSC differentiate into macrophages, lymphocytes, epithelial cells, neuronal cells, endothelial cells and hepatocytes. However, the Monocyte-Origin Multipotent Cells (MOMC) of the instant invention are much different from PSC of Zhao et al. in their properties, especially their differentiation abilities as demonstrated in Table 1 below.

TABLE 1.

	PSC	MOMC
Differentiation Abilities		
T-lymphocyte	+	-
epithelial cell	+	-
endothelial cell	+	+
neuronal cell	+	Culture under NGF stimulation (culture condition of PSC): - Coculture with rat neurons: +
hepatocyte	+	-
mesenchymal cell	not reported	+
proliferation from a single cell (cloning)	possible	impossible

The "+" and "-" signs show whether human MOMC cultured under the differentiation condition of PSC has a differentiation ability or not.

10. In view of the evidence presented above, there is a clear difference in differential abilities between MOMC and PSC. Furthermore, the cells of Zhao, et al. do not express vWF in the presence of EGF and do not differentiate into osteoblasts, skeletal myoblasts or chondrocytes under the differentiation induction conditions of MOMC. Therefore, one skilled in the art would conclude that MOMC is clearly distinct from said "cells that morphologically resembled fibroblasts" (Zhao, et al. page 2427, column 1, 3rd paragraph).

11. Thus, it is my experience and my opinion, as one skilled in the art of genetic engineering, that MOMC and PSC cells are not identical, in view of the differences in the differential abilities of these cells. These differences of differential abilities necessarily result in the differences of diseases for which these cells will be used as a transplant in the future. It is clear from these points that the instant invention could not be anticipated by the teachings of Zhao, et al.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Respectfully submitted,

Date : August 20, 2008

Masataka Kuwana